ARC • LNR

Excellence in Research and Development

AGRICULTURAL RESEARCH COUNCIL
PLANT PROTECTION RESEARCH INSTITUTE
P/Bag X134, Queenswood, Pretoria 0121

PROGRESS REPORT

SOIL ENZYME ANALYSES & COMMUNITY LEVEL PHYSIOLOGICAL PROFILING (BIOLOG ECOPLATE) – OTTOSDAL PROJECT

Ref: SM 080515 GSA

Prepared by: Johan Habig
Tel: (012) 808 8194 Fax: (012) 808 8299
Email: HabigJ@arc.agric.za

PREPARED FOR:
Client: Grain SA
Contact person: Dr Hendrik Smith
Tel: (012) 816 8039 Fax: 012 807 3166
Email: hendrik.smith@grainsa.co.za

22 MAY 2015

P stress and plant growth. Phosphatase activity has also been correlated with soil phosphorous state, with inorganic phosphorous having an inverse effect on phosphatase production. Phosphatase excreted by plant roots, has been reported to be exclusively acid phosphatase, which might be stimulated in cases of phosphorous deficiency, to increase availability of phosphate in the soil. Alkaline phosphatase, thus, would appear to be derived solely from soil microorganisms, allowing the distinction between acid phosphatase activity of roots and alkaline phosphatase activity of microorganisms. Urease activity plays a vital role in the regulation of N supply to plants, especially after urea fertilisation. Due to the influence of pH, temperature, organic matter content, and soil moisture on microbial enzymatic activity, they are considered early indicators of ecosystem stress and can act as biological indicators of soil degradation, compared to classical and slowly changing soil properties such as organic matter.

Materials and Methods

Determination of functional diversity

Whole-community substrate utilisation profiles (CSUP) are assessed when carbon sources are utilised. Soil samples were diluted in sterile distilled water and inoculated into Biolog EcoPlates™ (Biolog® Inc., Hayward, USA) containing 31 different carbon sources in each well (and one control well containing no carbon source), in triplicate. The plates were incubated at 28 °C. Utilisation of carbon sources by microbial populations reduced the tetrazolium dye inside the EcoPlate wells, causing a colour change which was measured twice daily over a period of 5-10 days at 590 nm to determine average well colour development (AWCD). The functional diversity of the soil microbial populations was determined using the amount and abundance of carbon substrates metabolised, as indicators of richness (Shannon-Weaver index) and evenness (Evenness index), respectively.

Determination of soil microbial enzymatic activity

The ability of the soil microbial population to obtain carbon, phosphorus and nitrogen, was assayed by measuring the ß-glucosidase, alkaline phosphatase, acid phosphatase, and urease activities in the soil. ß-Glucosidase and phosphatase activities were calculated by determining the release of *p*-nitrophenyl after the incubation of soil with *p*-nitrophenyl glucoside and *p*-nitrophenyl phosphate, respectively. Urease activity was determined where released ammonia was measured after the incubation of soil samples with a urea solution, where results were then calculated with reference to the calibration curve.

Statistical Analyses

Figure 1. Principal Component Analysis (PCA) ordination plot illustrating the differences in the average carbon source utilisation profiles between cover crop treatments sampled during April 2015.

Figure 1 demonstrates three main groups: Group 1 (blue circle) enclosing "skoon grond" and "veld" treatments; Group 2 (green circle) enclosing mainly treatments with "mielies"; and Group 3 (red circle) enclosing mainly treatments with "lablab".

Cluster analysis was performed as an alternative measure to visualise the groups illustrated in Figure 1. Dendograms were constructed to assign treatments into groups, so that treatments in the same cluster are more similar to each other, compared to treatments in other clusters. This is clearly illustrated by the dendogram in Figure 2.

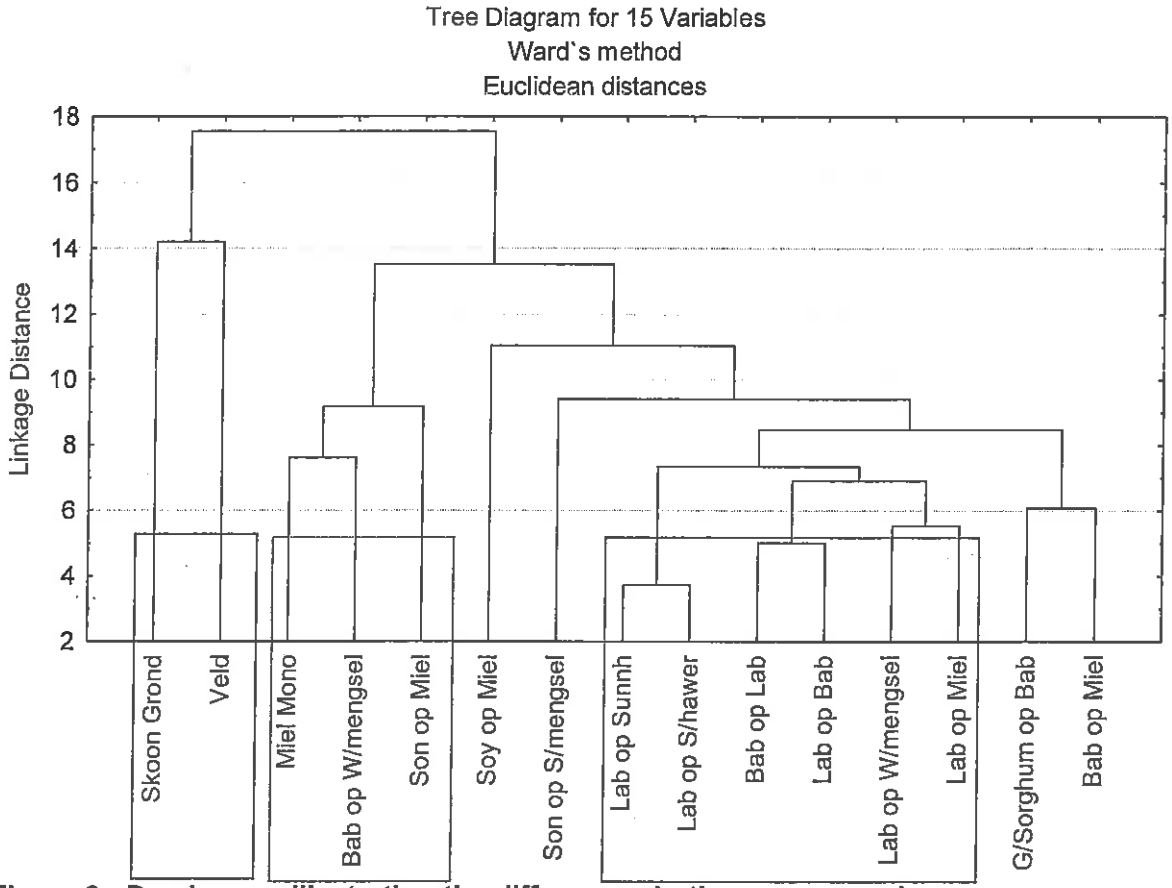


Figure 2. Dendogram illustrating the differences in the average carbon source utilisation profiles between cover crop treatments sampled during April 2015.

From the dendogram (Fig. 2), the CSUP of soil microbial communities found in Group 1 (blue block) clustered to the left, Group 2 (green block) clustered in the middle, and Group 3 (red block) clustered to the right. Since decomposing plant material on the soil surface (from the previous season) and the

carbon sources utilised by soil microbial communities in Biolog EcoPlates™, i.e., comparable to species richness in the soil. Values of the index typically range between 1.5 and 3.5, but rarely increase above 4.5. Depending on the treatments, a varying percentage of carbon sources were utilised, with values ranging from 1.53 to 2.72 as seen in Table 2.

The Evenness index, on the other hand, is used as an indicator of how abundant species are within a soil microbial community, i.e., how close in "numbers" each microbial species are in a soil microbial community. If abundances/quantities of different species in a community are measured, it will invariably be found that some species are rare, whereas others are more abundant/dominant. Substrate evenness assumes a value between 0 and 1, with 1 representing a situation in which all species are equally abundant within a microbial population present in the samples. This means less variation in microbial populations between species, thus, less dominance, and higher diversity. Substrate evenness indices obtained in this analysis ranged between 0.65 and 0.89 as seen in Table 2.

Table 2. The Shannon-Weaver diversity index and the Evenness Index between cover crop treatments sampled during April 2015.

Treatments	Shannon-Weaver Index	Evenness Index
Sonneblom op Somermengsel	2.16 ^{bcd}	0.76 ^{ab}
Babala op Mielies	2.30 ^{bcd}	0.79 ^{ab}
Soya op Mielies	1.85 ^{ab}	0.71 ^{ab}
Sonneblom op Mielies	2.03 ^{abc}	0.68 ^{a}
Lablab op Mielies	2.38 ^{bcd}	0.78 ^{ab}
Lablab op Swarthawer	2.55 ^{cd}	0.77 ^{ab}
Lablab op Wintermengsel	2.42 ^{bcd}	0.81 ^{ab}
Lablab op Sunnhemp	2.72 ^{d}	0.89 ^{b}
Babala op Wintermengsel	2.52 ^{cd}	0.78 ^{ab}
Graansorghum op Babala	2.35 ^{bcd}	0.74 ^{ab}
Lablab op Babala	2.55 ^{cd}	0.78 ^{ab}
Babala op Lablab	2.26 ^{bcd}	0.79 ^{ab}
Mielie Monokultuur	2.02 ^{abc}	0.75 ^{ab}
Veld	1.53 ^{a}	0.67 ^{a}
Skoon Grond	1.86 ^{ab}	0.65 ^{a}

*Means followed by similar letters do not differ significantly ($p > 0.05$).

According to the Shannon-Weaver Index, the number of active microbial species present in the "Lablab op Sunnhemp, "Lablab op swarthawer", "Lablab op Babala", and "Babala op Wintermengsel" treatments were higher compared to the number of active microbial species in the "Veld", Soya op mielies", and the "Skoon grond" treatments. The treatments containing Lablab

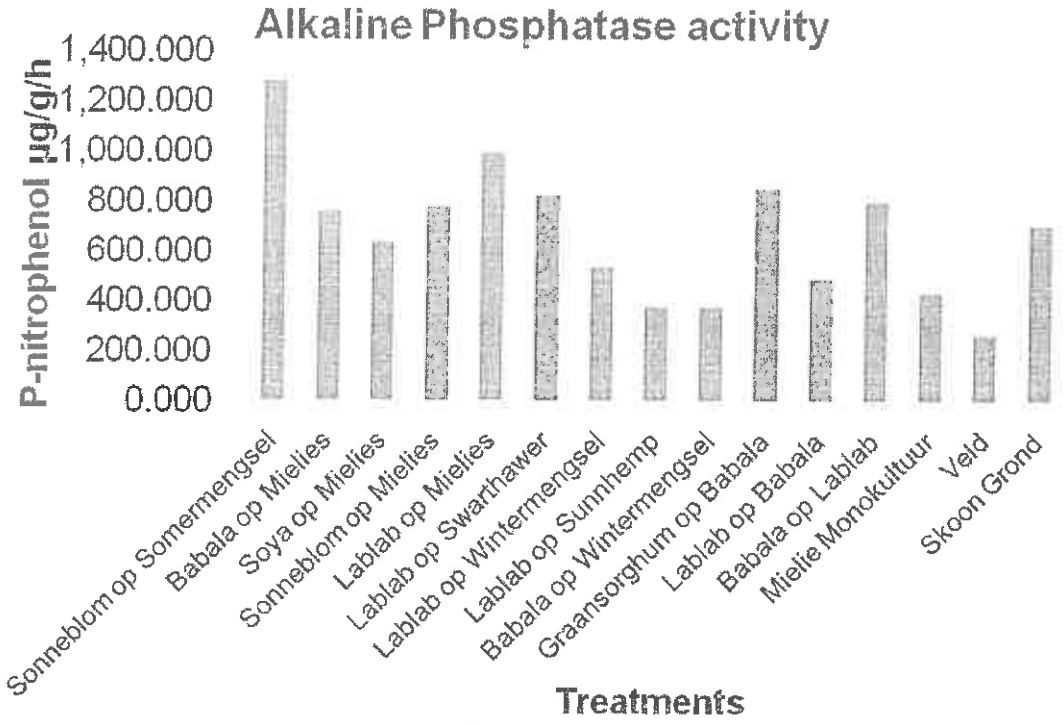
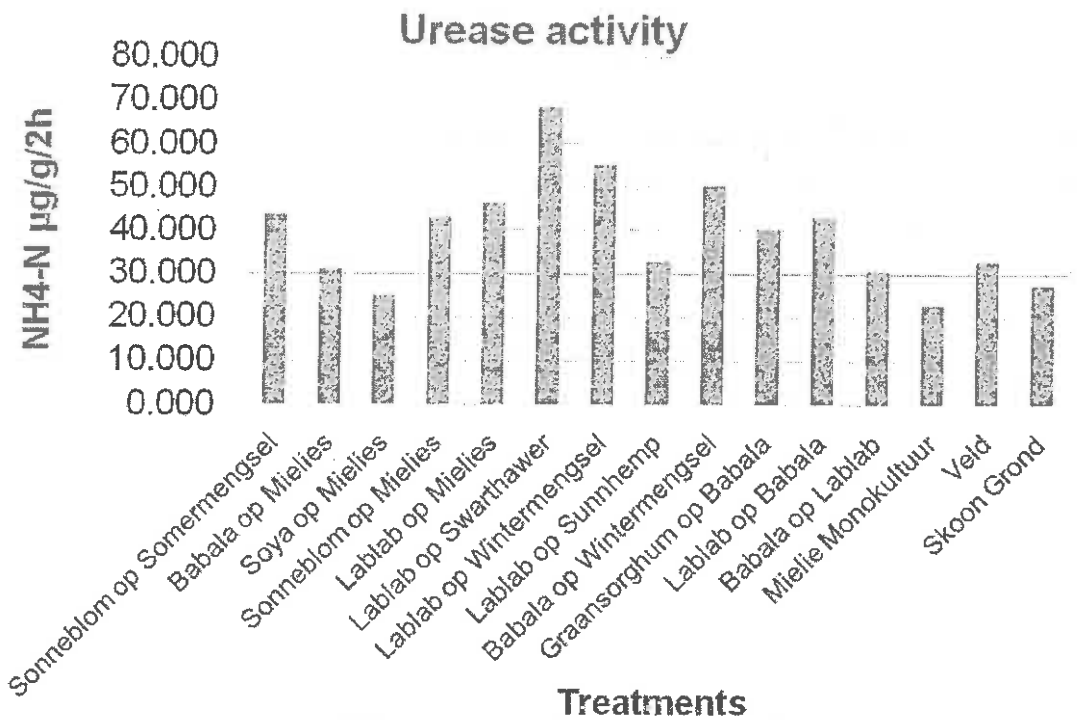


Figure 4. Bar graph illustrating soil microbial alkaline phosphatase activity in soils from the various cover crop treatments.



Graansorghum op Babala	1383.31 [j]	848.60 [i]	40.36 [f]
Lablab op Babala	1265.50 [g]	487.21 [d]	43.04 [g]
Babala op Lablab	1325.37 [h]	800.90 [j]	30.44 [d]
Mielie Monokultuur	726.81 [b]	433.70 [c]	22.83 [a]
Veld	649.94 [a]	263.40 [a]	32.77 [e]
Skoon Grond	1022.40 [c]	705.23 [g]	27.30 [c]

*Means followed by different letters differ significantly ($p < 0.05$).

Comparison of SolVita test with other biological soil quality tests

For the sake of interest, results from the SolVita test (SolVita colour chart reading, CO_2, and PMN) were compared with soil microbial diversity indices, microbial activity, and carbon source utilisation ("AWCD" in Figure 6 & 7).

Cluster analysis was performed and a dendogram constructed to cluster similar results together as seen in Figure 6.

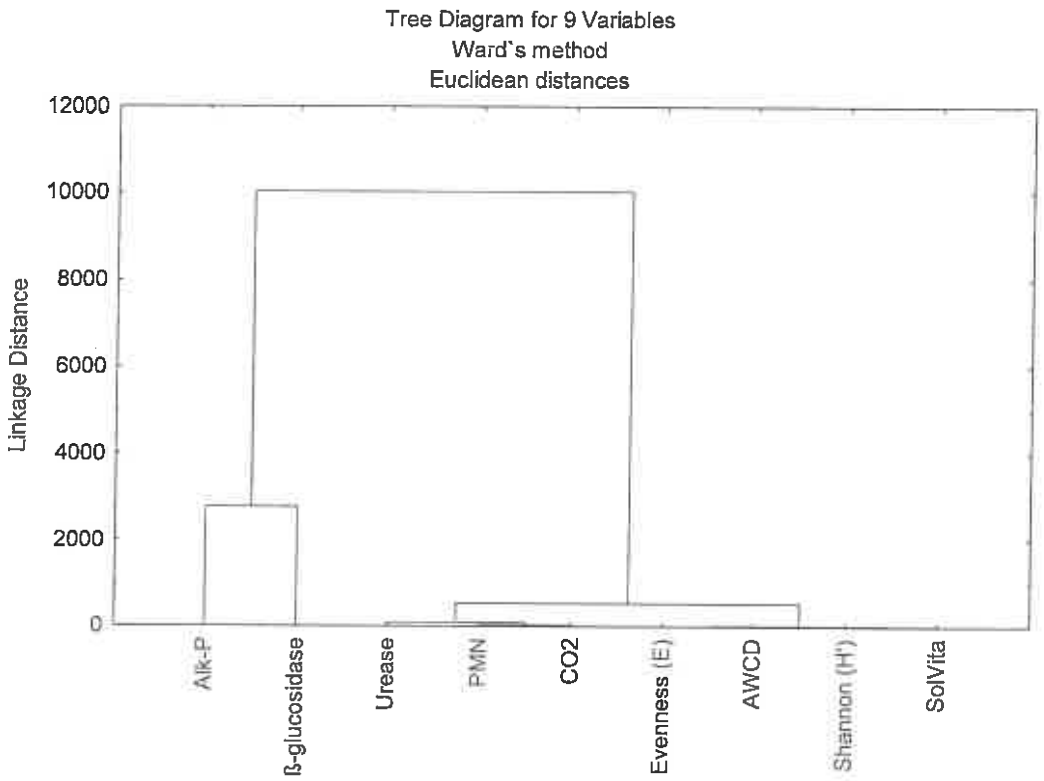


Figure 6. Dendogram illustrating the clustering of similar results from tests conducted on cover crop treatments sampled during April 2015.

Since differences in the tests clustering in the right (Figure 6), are not evident, the dendogram was magnified, showing more distinct clustering in Figure 7.

soil microbial activities, compared to treatments such as "veld" and "Mielie monokultuur".

The important role of cover crops in increasing soil quality is also clearly demonstrated in this study, with all the cover crop treatments demonstrating a higher soil microbial diversity and activity compared to natural veld. Not only is the planting of cover crops beneficial to soil life, the cropping sequence should also be carefully studied to guarantee the maximum increase in soil microbial diversity and activity, in the shortest possible time-frame.

Although carbon source utilisation profiles and soil enzymatic activities can be used as indicators of soil quality, these indicators are sensitive to changes in various environmental factors, crops planted, and soil and agricultural management practices. It is inadvisable to analyse composite soil samples, since it could give rise to statistical constraints, which, in turn, could result in limited analyses and incorrect conclusions. Integration and comparison of available data with more soil quality indicators, could contribute to more refined answers and possible predictions.

It is recommended that trends in carbon source utilisation profiles and enzymatic activity be monitored over time in order to attain a more complete reflection different agricultural practices might have on microbial diversity and activity as an indicator of soil fertility and health.

Acknowledgements

The Soil Microbiology Laboratory wishes to thank its extraordinary staff (Selina, Galaletsang, Johannes) for their extreme dedication that made all the analyses possible.